Effect of glutamine and spray-dried plasma on growth performance, small intestinal morphology, and immune responses of *Escherichia coli* K88$^+$-challenged weaned pigs[1,2]

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ABSTRACT: Forty weaned barrows (5.32 ± 0.3 kg BW) at 17 ± 2 d of age were used to investigate the effects of feeding glutamine and spray-dried plasma on the growth performance, small intestinal morphology, and immune responses of *Escherichia coli* K88$^+$-challenged pigs. Pigs were allotted to four treatments including: 1) nonchallenged control (NONC); 2) challenged control (CHAC); 3) 7% (as-fed basis) spray-dried plasma (SDP); and 4) 2% (as-fed basis) glutamine (GLN). On d 11 after weaning, all pigs were fitted with an indwelling jugular catheter. On d 12 after weaning, pigs in the CHAC, SDP, and GLN groups were orally challenged with skim milk *E. coli* K88$^+$ culture, whereas pigs in the NONC group were orally inoculated with sterilized skim milk. Rectal temperatures and fecal diarrheic scores were recorded and blood samples collected at 0 (baseline), 6, 12, 24, 36, and 48 h after the challenge for serum hormone and cytokine measurements. At 48 h postchallenge, all pigs were killed for evaluation of small intestinal morphology. There was no effect of feeding SDP or GLN on growth performance during the 11-d prechallenge period (P = 0.13). At 48 h after the challenge, CHAC pigs had decreased ADG (P = 0.08) and G:F (P = 0.07) compared with the NONC pigs; however, SDP and NONC pigs did not differ in G:F, and GLN and NONC pigs did not differ for ADG and G:F. At 6, 36, and 48 h after the challenge, CHAC, SDP, and GLN pigs had increased rectal temperature relative to the baseline (P = 0.09). At 12 and 36 h after the challenge, CHAC pigs had the highest incidence of diarrhea among treatments (P = 0.08). Serum IL-6 and ACTH were not affected by treatment or time after *E. coli* challenge (P = 0.11). In proximal, midjejunum, and ileum, CHAC pigs had greater villous atrophy and intestinal morphology disruption than NONC pigs (P < 0.01), whereas SDP and GLN pigs had mitigated villous atrophy and intestinal morphology impairment after *E. coli* challenge. Pigs in the SDP had the lowest GH at 12 h and the greatest GH at 36 h after the challenge among treatments (P = 0.08). Pigs in the NONC had the highest IGF-1 at 12 and 36 h postchallenge (P < 0.04). These results indicate that feeding glutamine has beneficial effects in alleviating growth depression of *E. coli* K88$^+$-challenged pigs, mainly via maintaining intestinal morphology and function, and/or possibly via modulating the somatotrophic axis.

Key Words: *Escherichia coli* K88$^+$, Glutamine, Immunity, Performance, Pigs, Spray-Dried Plasma

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Introduction

Glutamine (**GLN**) has important and unique metabolic functions, and it is considered a conditionally essential AA in some species under inflammatory conditions (Newsholme, 2001). The provision of GLN-enriched enteral or parenteral diets in various stress states associated with bacterial translocation decreases the incidence of translocation of bacteria by decreasing the adherence of bacteria to enterocyte (Souba et al., 1990). Glutamine-enriched the nutrition or 2 and 4%

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dietary supplementation decreased the mortality of i.p. *Escherichia coli*-challenged rats (Inoue et al., 1993). The positive effects of spray-dried plasma (**SDP**) on improving the growth performance of weaning pigs are well documented, especially during the first 2 wk after weaning (Van Dijk et al., 2001). Nollet et al. (1999) reported that feeding SDP decreased the excretion of pathogenic *E. coli* in the feces and provided protection against clinical symptoms of orally F18+ *E. coli*-challenged pigs. Bosi et al. (2004) reported that SDP improved the growth performance and protected against *E. coli* K88+ infection by maintaining intestinal mucosal integrity and modulating immune response of pigs.

Weaned pigs are regarded as a good model for studying the patho-physiology of diarrhea (Nabuurs, 1998), mainly because pigs are segregated from the passive immune protection of sow's milk. In addition, weaning stress, physical effects of solid diets, and other predisposing factors, such as rotavirus, increase their susceptibility to enterotoxigenic *E. coli* (**ETEC**) infection (Jones et al., 2001; Nabuurs, 1998). The objectives of this study were to investigate the effects of feeding GLN- or SDP-supplemented diets on growth performance and small intestinal morphology of weaned pigs challenged orally with ETEC K88+, and to explore the effect of nutritional modulation on the immunological, adrenal, and somatotrophic responses of early-weaned pigs after *E. coli* challenge.

Materials and Methods

Experimental Protocol

The animal protocol for this research was approved by the University of Missouri–Columbia Animal Care and Use Committee. Forty weaned barrows (17 ± 2 d of age, initial BW = 5.32 ± 0.3 kg) were obtained from a commercial pig farm in Missouri. Production records confirmed that the sows had not been exposed to *E. coli* vaccination. Pigs were allotted in a randomized complete block design with four treatments and ten blocks (blocked by initial BW). Treatments included 1) nonchallenged control (**NONC**, pigs fed a control diet and orally inoculated with sterilized skim milk); 2) challenged control (**CHAC**, pigs fed the same control diet and orally challenged with skim milk *E. coli* K88+ culture); 3) SDP treatment (pigs fed a 7% SDP [as-fed basis] diet and orally challenged with skim milk *E. coli* K88+ culture); and 4) GLN treatment (pigs fed a 2% GLN [as-fed basis] diet and orally challenged with skim milk *E. coli* K88+ culture). Treatment diet composition and nutrient profiles are shown in Table 1.

All pigs were fed treatment diets for the first week after weaning. During the first 7 d postweaning, one pig in the NONC died on d 2 of the study. In addition, two pigs in the CHAC, one pig in the NONC, and one pig in the GLN had very low appetite and lost more than 1 kg of BW, which was apparently not caused by treatment diets, and these four pigs were removed from the study. The remaining 35 pigs on trial were fed the treatment diets for four additional days from d 7 to 11 postweaning. On d 11 after weaning, all 35 pigs were fitted with an indwelling jugular catheter according to the procedures of Carroll et al. (1999). Feed intake and BW were recorded at 0600 of d 12 after weaning. At 0730 of d 12 after weaning, all pigs were bled via jugular catheter for serum baseline (0 h) measurement of ACTH, cortisol, GH, IGF-1, and IL-6. At 0800 of d 12, all pigs except those in the NONC were orally challenged with 5 mL of skim milk containing 5.5×10^8 cfu of *E. coli* K88+ by using a syringe attached to a polyethylene tube, whereas the pigs in the NONC were orally inoculated with an equal amount of sterilized

Table 1. Dietary composition and nutrient profile, % as-fed basis

Item	NONC and CHAC[a]	SDP[a]	GLN[a]
Ground corn	45.82	45.07	45.82
Soybean meal	20.00	18.35	20.00
Edible dried whey	20.00	20.00	20.00
Menhaden select fish meal	5.00	—	5.00
Blood cells	1.75	1.75	1.75
Spray-dried plasma	—	7.00	—
Corn oil	3.00	3.00	3.00
Cornstarch	2.00	2.00	—
Glutamine[b]	—	—	2.00
Limestone	0.50	1.09	0.50
Dicalcium phosphate	0.39	0.66	0.39
Salt	0.30	0.30	0.30
Vitamin premix[c]	0.25	0.25	0.25
Trace mineral premix[c]	0.15	0.15	0.15
L-Lysine·HCl	0.38	0.15	0.38
DL-Methionine	0.24	0.17	0.24
L-Threonine	0.22	0.06	0.22
Total	100	100	100
Calculated nutrient profile[d]			
CP, %	20.72	21.83	22.71
ME, kcal/kg	3,481	3,484	3,480
Total lysine, %	1.60	1.60	1.60
Total methionine + cystine, %	0.96	0.96	0.96
Total threonine, %	1.04	1.04	1.04
Total tryptophan, %	0.27	0.32	0.27
Total valine, %	1.05	1.17	1.05
Total isoleucine, %	0.81	0.80	0.81
Total calcium, %	0.80	0.80	0.80
Available phosphorous, %	0.40	0.40	0.40

[a]NONC (nonchallenged control) = pigs fed a control diet and orally inoculated with sterilized skim milk; CHAC (challenged control) = pigs fed the same control diet and orally challenged with *Escherichia coli* K88+; SDP treatment = pigs fed a 7% spray-dried plasma diet and orally challenged with *E. coli* K88+; GLN treatment = pigs fed a 2% glutamine diet and orally challenged with *E. coli* K88+.

[b]Provided by Ajinomoto Co., Inc., Tokyo, Japan.

[c]Mineral and vitamin mix provided per kilogram of diet: vitamin A, 11,000 IU; cholecalciferol, 1,100 IU; vitamin E, 22 IU; menadione, 4 mg; riboflavin, 8.25 g; niacin, 33 mg; d-pantothenic acid, 28 mg; vitamin B_{12}, 0.03 mg; 16.5 mg of Cu from $CuSO_4 \cdot 5H_2O$; 165 mg of Zn from $ZnSO_4 \cdot 7H_2O$; 33 mg of Mn from $MnSO_4 \cdot 5H_2O$; 165 mg of Fe from $FeSO_4 \cdot 5H_2O$; 0.3 mg of I from KI; 0.3 mg of Se from $Na_2SeO_3 \cdot 5H_2O$; and Santoquin, 50 mg.

[d]Calculated based on the feed ingredient composition data from our lab and from manufacturers.

skim milk as a placebo. The K88$^+$ ETEC was isolated from a North Carolina farm by Rollins Animal Disease Diagnostic Laboratory, North Carolina State University (Log No. 25612-96). The ETEC was confirmed by PCR genotyping as possessing the genes necessary for toxin expression in vitro, which was tested to be ETEC expressing K88 fimbrial antigen, and positive for heat-labile toxin LT and heat-stable toxin STb (**LT/STb$^+$**). The isolated K88$^+$ LT/STb$^+$ *E. coli* was then sent to the ARS-USDA laboratory at the University of Missouri–Columbia and cultured for challenge. The primary cultures of ETEC strain were grown in Bacto tryptic soy broth (Fisher Scientific, Fair Lawn, NJ) at 37°C for 24 h using 1% inoculum volume from stocks stored at –20°C in 30% glycerol. The Bacto tryptic soy broth was vortexed with purified water at 30 g/L and autoclaved before inoculating. The ETEC K88$^+$ strain for oral challenge was prepared by inoculating skim milk using 1% inoculum volume with stock of Bacto tryptic soy broth culture and incubated at 37°C with shaking for 24 h. The quantification of the inoculum was using the lauryl tryptose broth (Difco, Becton Dickinson and Co., KS) for incubation at 37°C for 24 h and the most-probable-number test method for microbial count with 10-fold dilutions and five tubes per dilution to quantify the *E. coli* population of skim milk culture, which was tested to be 1.1×10^8 cfu/mL. At 1400 (6 h) and 2000 of d 12 (12 h), 0800 (24 h), and 2000 (36 h) of d 13, and 0800 (48 h postchallenge) of d 14 after weaning, all pigs were bled again via jugular catheter for serum hormone and cytokine analysis. At each time point, fecal scores (1 = normal, solid feces; 2 = soft, looser than normal feces, slight diarrhea; 3 = moderate diarrheic feces; 4 = liquid, severe diarrheic feces) and rectal temperatures were recorded for all pigs. Pigs were maintained in individual pens of an environmentally controlled segregated nursery barn with ad libitum access to mash feed and water.

Small Intestinal Morphology

At 0900 on d 14 after weaning, all pigs were killed by captive bolt followed by exsanguination for collection of intestinal segments. The small intestine was dissected free of mesenteric attachment and placed on a smooth surface. Three 5-cm segments at 25, 50, and 75% of the total intestinal length were removed from the intestine and referred to proximal jejunal, midjejunal, and ileal segment, respectively. The intestinal segments were stored in 10% neutral buffered formalin for 24 h, after which they were cut and histological slides were prepared. Three cross sections (5 μm thick) of each intestinal segment were processed in low-melt paraffin and stained with hematoxylin and eosin. Intestinal morphological measurements included the following criteria: villus height, crypt depth, villus area, and villus volume. These criteria were quantified by using a digitized board coupled to a video monitor receiving output from a video camera mounted on a binocular microscope. Output from the digitized board was collected with the program Image-Pro Plus (Silver Spring, MD). The 10 longest and straightest villi and their associated crypts were measured. Mean values of villus height, crypt depth, villus area, villus volume, and villus height:crypt depth ratio within each segment were calculated for statistical analyses. More details were described in our previous publication of Touchette et al. (2002).

Serum Hormone and Cytokine Analyses

Serum concentration of ACTH was determined by using a commercially available human ACTH RIA kit (DSL-2300; Diagnostic Systems Laboratories, Inc., Webster, TX), which we have previously validated for pigs (Daniel et al., 1999). Minimum detection limit of this assay is 3.5 pg/mL, with an intraassay CV of 4.0%. Serum concentration of cortisol was determined using a Coat-a-Count assay kit (Diagnostic Products Corp., Los Angeles, CA), which we have also previously validated in pigs (Daniel et al., 1999). Minimum detection limit was 2 ng/mL, with an intraassay CV of 3.0%. Serum concentration of GH was determined by using a commercially available porcine GH RIA kit (Linco Research, Inc., St. Charles, MO). The minimum detection limit was 1 ng/mL, with an intraassay CV of 4.0%. Serum concentration of IGF-1 was determined by using a commercially available porcine IGF-1 IRMA kit (DSL-2800, Diagnostic Systems Laboratories, Inc.). The minimum detection limit was 2 ng/mL, with an intraassay CV of 3.9%. Serum concentration of IL-6 was determined by a commercially available porcine ELISA kit (Quantikine P6000; R&D Systems, Inc., Minneapolis, MN). The minimum detection limit of porcine IL-6 for this kit is typically 10 ng/mL, with an intraassay CV of 2.2%. All analyses were conducted as outlined by the manufacturer.

Statistical Analyses

All the data for the growth performance and intestinal morphological criteria were subjected to ANOVA appropriate for randomized complete block design by using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Statistical difference of treatment means comparisons were made with Fisher's protected LSD. Treatment and block were considered the main effects. All data for the rectal temperature, fecal scores, serum ACTH, cortisol, IL-6, GH, and IGF-1 were analyzed with the Proc Mixed procedure of SAS as a randomized complete block design with repeated measures over time on each experimental unit (individual pens; Littell et al., 1998). The model included terms for the fixed effects of treatment, time, and treatment × time interaction, and blocks and pens were considered random effects. Comparisons between treatments and/or sampling times were made by using the Slice option only when a significant ($P < 0.10$) F-test for the main effect or interaction was found with the Proc Mixed model

Table 2. Effects of dietary spray-dried plasma and glutamine on the growth performance of pigs during pre- and postchallenge periods

Item	NONC	CHAC	SDP	GLN	SEM	Main effect *P*-value
No. of pigs	8	8	10	9		
BW, kg						
d 0	5.30	5.43	5.29	5.30	0.13	0.27
d 11	8.37	8.46	7.88	8.13	0.43	0.13
d 14	9.22	8.87	8.45	8.77	0.48	0.10
Days 0 to 11 (prechallenge)						
ADG, g	279	275	235	257	19.11	0.30
ADFI, g[c]	341	345	307	314	19.98	0.42
G:F	0.82	0.80	0.77	0.82	0.03	0.44
Days 12 to 14 (postchallenge)[d]						
ADG, g	424[a]	206[b]	285[b]	319[ab]	48.91	0.08
ADFI, g[c]	483	451	436	413	29.01	0.52
G:F	0.86[a]	0.44[b]	0.66[ab]	0.73[a]	0.09	0.07
Days 0 to 14 (prechallenge and postchallenge)[d]						
ADG, g	302	264	243	266	18.15	0.14
ADFI, g[c]	363	361	327	329	18.41	0.33
G:F	0.83	0.73	0.74	0.80	0.03	0.11

[a,b]Means in a row without a common superscript differ, $P = 0.08$. NONC (nonchallenged control) = pigs fed a control diet and orally inoculated with sterilized skim milk; CHAC (challenged control) = pigs fed the same control diet and orally challenged with *Escherichia coli* K88+; SDP treatment = pigs fed a 7% spray-dried plasma diet and orally challenged with *E. coli* K88+; GLN treatment = pigs fed a 2% glutamine diet and orally challenged with *E. coli* K88+.

[c]ADFI is on an as-fed basis.

[d]Pigs in the CHAC, SDP, and GLN treatments were orally challenged with 5.5×10^8 cfu *E. coli* K88+ at 0800 of d 12, and all pigs were killed at 0900 of d 14; therefore, the postchallenge growth performance was counted for 2 d only, and overall growth performance (prechallenge and postchallenge period) was counted for 13 d only.

analysis of repeated measures. An alpha level of $P < 0.10$ was used as the criterion for statistical significance.

Results

Growth Performance

The growth performance data are presented in Table 2. There was no difference in initial BW ($P = 0.27$) among treatments. During d 0 to 11 after weaning, there was no difference in BW, ADG, ADFI, and G:F among treatments ($P = 0.13$). Within 48 h of oral *E. coli* K88+ challenge (d 12 to 14 after weaning), CHAC pigs had decreased ADG ($P = 0.08$) and G:F ($P = 0.07$) compared with the NONC pigs. Moreover, compared with the NONC pigs, the SDP pigs had decreased ADG ($P = 0.08$), but comparable G:F. However, within 48 h, *E. coli*-challenged pigs in the GLN group and pigs in the NONC group without *E. coli* infection did not differ in ADG and G:F. During the 14-d study, there was no difference among treatments in overall ADG, ADFI, and G:F ($P = 0.11$).

Rectal Temperature and Fecal Score

To accurately track *E. coli* K88+-induced enteric disease, rectal temperature (Figure 1) and fecal scores (Figure 2) were measured at 0 (baseline), 6, 12, 24, 36, and 48 h after the challenge. During the 48 h following

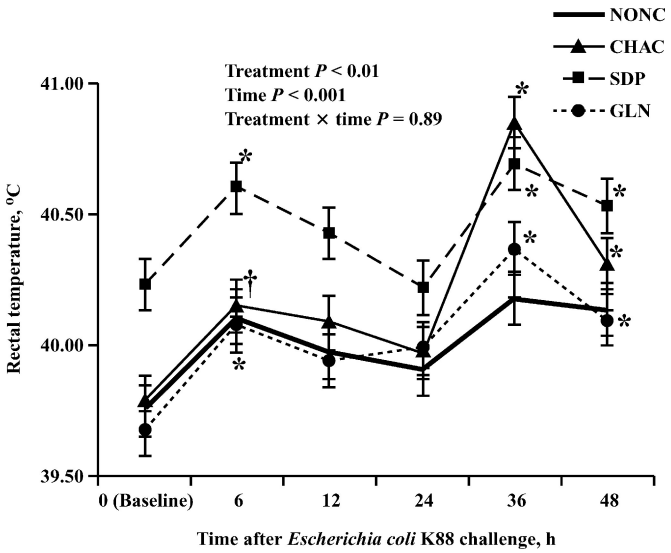

Figure 1. Rectal temperature of the NONC (nonchallenged control, n = 8), CHAC (*Escherichia coli*-challenged control, n = 8), SDP (*E. coli*-challenged pigs fed a 7% spray-dried plasma diet, n = 10), and GLN (*E. coli*-challenged pigs fed a 2% glutamine diet, n = 9) pigs during 48 h following infection with *E. coli* K88+. †*Denote temperatures greater than those at baseline, $P = 0.09$ and $P < 0.05$, respectively.

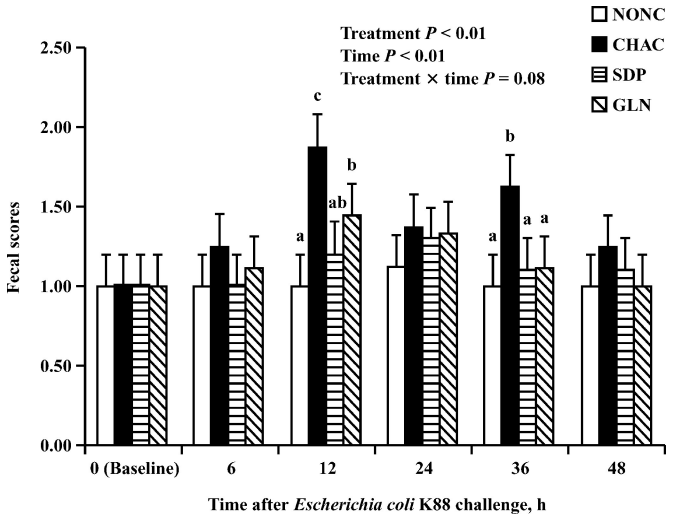

Figure 2. Fecal scores of the NONC (nonchallenged control, n = 8), CHAC (*Escherichia coli*-challenged control, n = 8), SDP (*E. coli*-challenged pigs fed a 7% spray-dried plasma diet, n = 10), and GLN (*E. coli*-challenged pigs fed a 2% glutamine diet, n = 9) pigs during 48 h following infection with *E. coli* K88$^+$. Within 48 h after the challenge, bars without common letters differ, $P = 0.08$.

E. coli challenge, there was a treatment ($P < 0.01$) and time effect ($P < 0.001$), but no treatment × time interaction for rectal temperature ($P = 0.89$). Compared with the baseline rectal temperature, there was no increase of rectal temperature for the NONC pigs within 48 h after inoculation with sterilized skim milk ($P = 0.11$); however, CHAC pigs had increased rectal temperature at 6 ($P = 0.09$), 36, and 48 h ($P < 0.05$) after the challenge with *E. coli* relative to the baseline. Pigs in the SDP and GLN had increased rectal temperature at 6, 36, and 48 h ($P < 0.05$) following *E. coli* challenge compared with the baseline.

During the 48 h following *E. coli* K88$^+$ challenge, there was a treatment × time interaction ($P = 0.08$) for fecal scores (Figure 2). At 12 h postchallenge, CHAC pigs had the highest incidence of diarrhea among treatments, and GLN pigs had higher fecal scores than NONC pigs ($P = 0.08$). Pigs in the CHAC group had higher fecal scores than the NONC, SDP, and GLN pigs at 36 h following oral pathogenic infection ($P = 0.08$).

Small Intestinal Morphology

The small intestinal morphological criteria were measured within 48 h following oral *E. coli* challenge (Table 3). In all three small intestinal segments, there was no difference in crypt depth among treatments ($P = 0.15$). In the proximal jejunum, pigs in the CHAC had decreased villus height, villus height:crypt depth ratio, villus area, and villus volume ($P < 0.01$) compared with pigs in the NONC. Compared with the NONC pigs, SDP pigs had lower villus volume ($P < 0.01$), whereas villus height, villus height:crypt depth ratio, and villus area were not different. However, GLN pigs maintained the overall intestinal integrity of proximal jejunum relative to those in the NONC.

In the midjejunum, the CHAC pigs had decreased villus height ($P = 0.08$), villus height:crypt depth ratio, villus area, and villus volume ($P < 0.01$) compared with the NONC pigs. Pigs in both the SDP and GLN groups maintained the overall intestinal integrity of midjejunum compared with the NONC pigs. Moreover, GLN pigs had even higher villus height:crypt depth ratio relative to those in the NONC group ($P < 0.01$).

In the ileum, following a pattern similar to that seen with the proximal and midjejunum, CHAC pigs had decreased villus height, villus height:crypt depth ratio, villus area, and villus volume ($P < 0.01$). The SDP and GLN pigs maintained the overall intestinal integrity of the ileum and had similar villus height, villus area, and villus volume compared with the NONC pigs. Moreover, pigs in the SDP and GLN had even higher villus height:crypt depth ratio relative to those in the NONC ($P < 0.01$).

Serum Hormone and Cytokines

Serum ACTH was not affected by treatment ($P = 0.11$) or time ($P = 0.14$) following *E. coli* K88$^+$ challenge, and there was no treatment × time interaction ($P = 0.92$; data not shown; overall mean 68.7 ± 4.3 pg/mL). Although there was no treatment × time interaction ($P = 0.36$) or treatment effect ($P = 0.28$), there was still a time effect ($P = 0.09$), in which pigs across treatment had the lowest cortisol levels at 24 h after the challenge with *E. coli* (Figure 3). Serum IL-6 was not affected by treatment ($P = 0.42$) or time ($P = 0.28$) within 48 h following *E. coli* infection, and there was no treatment × time interaction ($P = 0.12$; data not shown; overall mean 72.1 ± 14.8 pg/mL).

There was a treatment × time interaction for serum GH ($P = 0.08$) within 48 h following the *E. coli* challenge. Between 0 and 6 h postchallenge, pigs in all treatments had increased GH relative to the baseline ($P < 0.001$). Pigs in the NONC and GLN groups had greater ($P = 0.08$) circulating GH levels than did pigs in the SDP at 6 h postchallenge (Figure 4). By 24 h postchallenge, GH for all treatments had gradually returned to the preinfection baseline levels. At 36 h postchallenge, there was a resurgence of increasing circulating GH concentration for pigs in the SDP, which was higher than that for pigs in the NONC and CHAC groups ($P = 0.08$). By 6 h postchallenge, IGF-1 for all treatments was reduced similarly ($P < 0.001$), although the NONC pigs were not orally challenged with *E. coli* (Figure 5). However, within 12 h after inoculation, IGF-1 of the NONC pigs without *E. coli* infection was restored to the baseline level and was higher than the IGF-1 concentrations for *E. coli* K88$^+$-challenged pigs in the CHAC, SDP, and GLN groups ($P < 0.04$). At 36 h postchallenge with *E. coli* K88$^+$, SDP pigs had lower serum concentrations of IGF-1 than NONC pigs ($P < 0.04$).

Table 3. Effects of dietary spray-dried plasma and glutamine on intestinal morphology of weaning pigs after 48 h oral *Escherichia coli* K88+ challenge

	NONC	CHAC	SDP	GLN	SEM	Main effect *P*-value
No. of pigs	8	8	10	9		
Proximal jejunum[d]						
Villus height, μm	319[a]	230[b]	295[a]	297[a]	5.52	<0.01
Crypt depth, μm	94	88	88	82	2.09	0.22
Villus height:crypt depth ratio	3.49[a]	2.72[b]	3.54[a]	3.87[a]	0.11	<0.01
Villus area, mm^2	0.020[a]	0.012[b]	0.018[a]	0.018[a]	0.0004	<0.01
Villus volume, mm^3	0.0014[a]	0.0007[c]	0.0011[b]	0.0012[ab]	0.00005	<0.01
Midjejunum[d]						
Villus height, μm	296[a]	224[b]	288[a]	284[a]	7.26	0.08
Crypt depth, μm	90	93	82	76	2.81	0.15
Villus height:crypt depth ratio	3.36[b]	2.57[c]	3.71[ab]	4.01[a]	0.12	<0.01
Villus area, mm^2	0.018[a]	0.011[b]	0.017[a]	0.017[a]	0.0005	<0.01
Villus volume, mm^3	0.0013[a]	0.0007[b]	0.0012[a]	0.0011[a]	0.00006	0.01
Ileum[d]						
Villus height, μm	251[a]	187[b]	253[a]	248[a]	6.76	<0.01
Crypt depth, μm	91	94	84	80	2.31	0.15
Villus height:crypt depth ratio	2.84[b]	2.07[c]	3.12[a]	3.19[a]	0.05	<0.01
Villus area, mm^2	0.016[a]	0.009[b]	0.014[a]	0.015[a]	0.001	<0.01
Villus volume, mm^3	0.0011[a]	0.0005[b]	0.0009[a]	0.0009[a]	0.00006	0.01

[a,b,c]Means in a row without a common superscript differ, $P = 0.08$. NONC (nonchallenged control) = pigs fed a control diet and orally inoculated with sterilized skim milk; CHAC (challenged control) = pigs fed the same control diet and orally challenged with *Escherichia coli* K88+; SDP treatment = pigs fed a 7% spray-dried plasma diet and orally challenged with *E. coli* K88+; GLN treatment = pigs fed a 2% glutamine diet and orally challenged with *E. coli* K88+.

[d]Proximal jejunum, midjejunum, and ileum samples were taken at 25%, 50%, and 75% of total length of the small intestine, respectively.

Within 48 h following oral pathogen infection, CHAC and SDP pigs had lower IGF-1 levels relative to the preinfection baseline ($P = 0.06$), whereas NONC and GLN pigs maintained serum IGF-1 concentrations to the preinfection baseline.

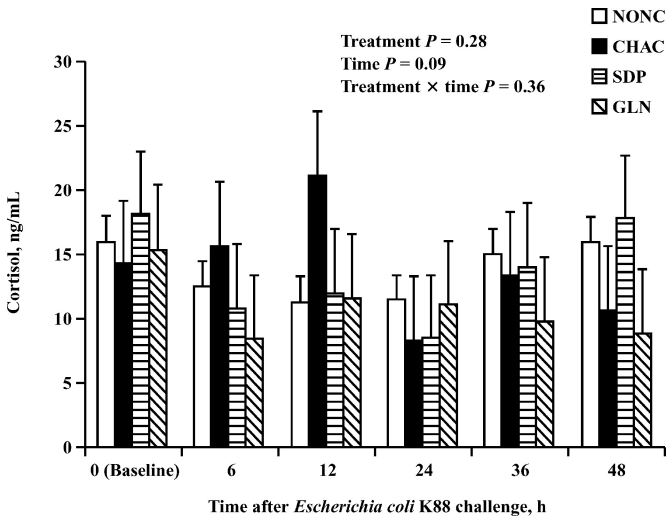

Figure 3. Serum cortisol of the NONC (nonchallenged control, n = 8), CHAC (*Escherichia coli*-challenged control, n = 8), SDP (*E. coli*-challenged pigs fed a 7% spray-dried plasma diet, n = 10), and GLN (*E. coli*-challenged pigs fed a 2% glutamine diet, n = 9) pigs during 48 h following infection with *E. coli* K88+.

Discussion

Enterotoxigenic *E. coli* strains that express K88 fimbrial antigens are a major cause of diarrhea and death of neonatal and weaned pigs for swine producers (Francis et al., 1998). During the d 0 to 11 prechallenge period, feeding 2% GLN diet or 7% SDP diet did not improve growth, feed intake, or feed efficiency relative to the corn-soy-whey-fish meal control diet. In a previous study, we found that, compared with a corn-soy-lactose-fish meal diet, feeding 3.5% SDP improved ADG during the first week after weaning (Yi et al., 2002); however, Carroll et al. (2002) reported that pigs fed 7% SDP had similar BW to pigs fed a diet without SDP during the first week after weaning. Coffey and Cromwell (1995) found that SDP addition to weaning pig diets improved growth and feed intake under a less hygienic environment, but had no effect on feed intake and weight gain in an experimentally controlled environment with minimal antigen exposure. In the current study, pigs were individually raised under a hygienic, thermo-controlled environment, which might be the reason why there was no beneficial effect of feeding SDP on growth performance. Compared with the control diets, feeding 2% GLN diet during the first 11 d after weaning did not result in an improvement in ADG and G:F, which agrees with our previous findings (Yi et al., 2002). In contrast to our findings, Lackeyram et al. (2001) observed that 0.8% GLN supplementation in corn-soy-based diets was effective in enhancing BW gain of early-weaned, 10-d-old pigs during a 12-d study.

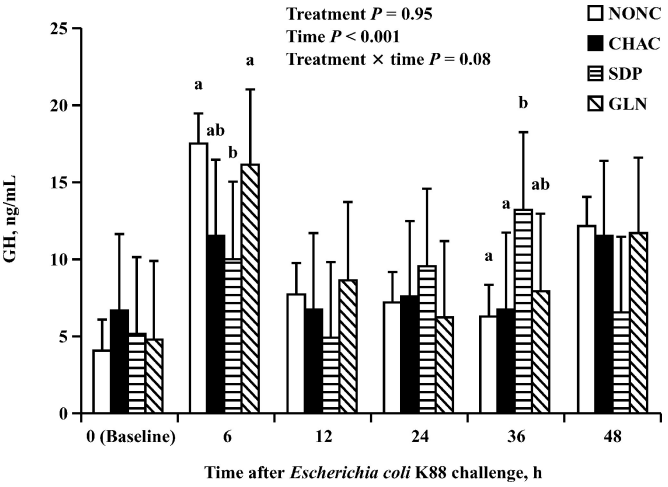

Figure 4. Serum GH of the NONC (nonchallenged control, n = 8), CHAC (*Escherichia coli*-challenged control, n = 8), SDP (*E. coli*-challenged pigs fed a 7% spray-dried plasma diet, n = 10), and GLN (*E. coli*-challenged pigs fed a 2% glutamine diet, n = 9) pigs during 48 h following infection with *E. coli* K88$^+$. Within 48 h after the challenge, bars without common letters differ, $P = 0.08$.

During 48 h postchallenge with *E. coli* K88$^+$, CHAC pigs had more than a 50% decrease in ADG and approximately a 49% decrease in G:F compared with NONC pigs without *E. coli* infection. However, the feed intake of CHAC pigs was not affected after *E. coli* challenge, indicating that the growth depression might be caused by other factors, such as disrupted intestinal integrity and function and impaired digestion and absorption

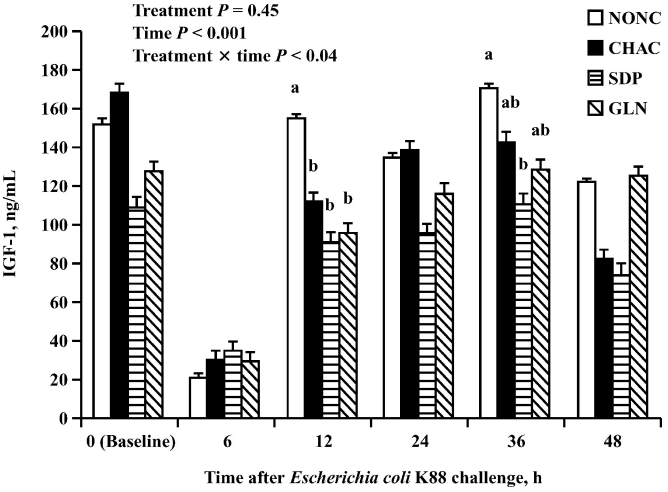

Figure 5. Serum IGF-1 of the NONC (nonchallenged control, n = 8), CHAC (*Escherichia coli*-challenged control, n = 8), SDP (*E. coli*-challenged pigs fed a 7% spray-dried plasma diet, n = 10), and GLN (*E. coli*-challenged pigs fed a 2% glutamine diet, n = 9) pigs during 48 h following infection with *E. coli* K88$^+$. Within 48 h after the challenge, bars without common letters differ, $P < 0.04$.

capability. Within 48 h of pathogen infection, SDP and NONC pigs did not differ in G:F, although the ADG of SDP pigs was decreased relative to the NONC pigs. In contrast to our findings, the beneficial effects of SDP relative to other protein sources in improving the growth performance of early-weaned pigs orally challenged with *E. coli* K88$^+$ were reported by Bosi et al. (2001, 2004). The more effective protection against *E. coli* K88$^+$ infection in their studies might be attributed to higher *E. coli* challenge dosage, relatively less hygienic environment, as well as a higher dietary SDP concentration. We observed no difference in ADG and G:F between NONC and GLN pigs. The beneficial effects of GLN in alleviating the growth depression and feed utilization inefficiency of *E. coli* K88$^+$-challenged pigs indicated the importance of exogenous GLN supply. It has also been reported that GLN might act as a metabolic regulator to enhance protein synthesis and decrease protein catabolism under infection and inflammation (Lobley et al., 2001). Therefore, it is possible that the 2% GLN diet mitigated growth suppression associated with *E. coli* K88$^+$ challenge by decreasing protein catabolism and maintaining protein deposition rate of skeletal muscle.

We noted that CHAC, SDP, and GLN pigs had febrile responses at 6, 36, and 48 h postchallenge with *E. coli* K88$^+$. However, there was no febrile response of pigs in the NONC group without *E. coli* infection. In agreement with our observations, Hamback et al. (2002) found increased rectal temperatures of weanling pigs within 24 h following an oral *E. coli* K99$^+$ challenge. Yoo et al. (1997) reported that after an i.p. *E. coli* (serotype 078) challenge, weaned pigs had elevated rectal temperatures during d 5 to 7 postchallenge. In contrast to our findings, Van Dijk et al. (2002) did not observe a rectal temperature elevation in pigs fed either an 8% SDP diet or a control diet without SDP within 7 d following an oral *E. coli* (O139:K82 LT$^-$) challenge. The different febrile responses across these studies might be affected by different routes of pathogen administration, administration dose, and strains of *E. coli*. Within 48 h postinoculation with sterilized skim milk, pigs in the NONC without *E. coli* challenge had no severe incidence of diarrhea, indicating there was no ETEC K88$^+$ colonization and proliferation in the small intestine; however, at 12 h postchallenge with *E. coli* K88$^+$, there was an outbreak of diarrhea in 16 pigs, mainly from the CHAC and GLN groups. Pigs in the CHAC had a higher incidence of diarrhea as confirmed by the fecal scores. Pigs in the SDP seemed to be protected against the scouring. We observed that after 24 h postchallenge, most of the pigs with diarrhea at 12 h recovered and did not show apparent diarrheic symptoms. At 36 h postchallenge, there was a reoccurrence of pig scours, and more pigs had diarrhea compared to 24 h, although it was not as widespread as at 12 h after the challenge. However, only CHAC pigs had higher fecal scores relative to the NONC pigs at 36 h postchallenge. Enterotoxins produced by ETEC bind to the intestinal epithelial recep-

tors and initiate downstream intracellular reactions, leading to severe secretory diarrhea, especially during the first 5 to 10 d after weaning (Hampson et al., 2001). In agreement with our findings, Jones et al. (2001) found that fecal shedding of ETEC K88$^+$ was observed immediately following an oral *E. coli* K88$^+$ infection and reached a maximum at approximately 2 d. After 12 h postchallenge, we observed that the highest number of pigs showed clinical symptoms of diarrhea and vomiting, indicating the *E. coli* K88$^+$ colonization in the intestinal enterocyte and enterotoxin secretion. Similarly, Van Dijk et al. (2002) reported that there was a rapid increase of the fecal scores in the first 2 d after an orally pathogenic *E. coli* (O139:K82 LT$^-$) infection in pigs fed both SDP- and non-SDP-supplemented diets. However, CHAC pigs had a fluctuating pattern of fecal scores within 48 h postchallenge, which might be a reflection of fecal *E. coli* K88$^+$ shedding and reinfection. After enterotoxin infection of intestinal brush border, decreased small intestinal disaccharidase activity results in retention of disaccharides (primarily lactose) in the lumen of the small intestine, which might cause osmotic diarrhea (Graham et al., 1984). Therefore, it is also possible that the reoccurring scours of CHAC pigs at 36 h after the challenge might be caused by disruption of intestinal integrity and function, and hence retention of undigested nutrients and subsequent osmotic diarrhea. The positive effect of feeding SDP in alleviating the severity and incidence of diarrhea caused by *E. coli* infection at 12 and 36 h postchallenge in this study was also reported before. In agreement with our findings, Nollet et al. (1999) reported that feeding SDP decreased excretion of ETEC in the feces and provided protection against clinical symptoms of orally *E. coli* F18$^+$ challenged weaned pigs. The beneficial effects of feeding 2% GLN on mitigating pig diarrhea also was observed within 12 and 36 h following oral *E. coli* K88$^+$ challenge. There may be several contributing factors. First, GLN is a potential precursor for glycoprotein *N*-acetylglucosamine and *N*-acetylgalactosamine synthesis (Reeds and Burrin, 2001). Anderson et al. (1980) found that *E. coli* K88$^+$ binding to porcine intestinal brush border membrane was partially inhibited by glycoprotein with terminal *N*-acetylglucosamine and *N*-acetylgalactosamine residues. Secondly, GLN may be a key factor in maintaining mucosal structure, especially in the maintenance of tight junctions and permeability of intestinal mucosa (Panigrahi et al., 1997). Thirdly, an enteral GLN source may affect intestinal NaCl, water, and electrocyte absorption and homeostasis. Glutamine was reported to enhance Na^+ transport at both the villus tip and crypt cells of orally *E. coli* K99$^+$-challenged calves (Brooks et al., 1997).

Rose et al. (1987) reported that heat-stable STb secreted by ETEC infection is capable of causing partial villus atrophy in young pigs. In the current study, it is clear that after *E. coli* K88$^+$ LT/STb$^+$ challenge, compared with NONC pigs without *E. coli* infection, CHAC pigs had decreased villus height and decreased villus area and volume. However, the SDP, GLN, and NONC pigs did not differ in intestinal morphology, indicating the beneficial effects of SDP and GLN in alleviating villus atrophy and attenuating the decrease in small intestinal absorptive area and volume. In agreement with our findings, Bosi et al. (2004) reported that SDP maintained intestinal mucosal integrity and improved growth performance of orally *E. coli* K88$^+$-infected pigs. The components of SDP, such as epidermal growth factor and IGF may be trophic to the small intestinal villi. In addition, the high content of GLN plus glutamate (about 11.2%; Van Dijk et al., 2001) and immunoglobulin (approximately 25%; Touchette, 1999) in SDP may contribute to the mitigation of villus atrophy and intestinal morphology disruption. In a previous study, we found that feeding a 3.5% SDP diet increased jejunal villus height, whereas feeding a 1.2% GLN diet did not improve small intestinal morphology during the first week after weaning (Yi et al., 2002). However, Liu et al. (2002) reported that compared with a corn-soy-whey diet, feeding 28-d-old weaned pigs a 1% GLN diet prevented jejunal atrophy during the first wk after weaning and improved the capability of small intestinal absorption on d 7 and 14 after weaning. Mitigation of villus atrophy and the lack of overall disruption of intestinal morphology of infected pigs in this study may accentuate the increased GLN requirement of intestinal mucosa under *E. coli* challenge situations, especially utilization of the GLN amide group for purine and pyrimidine synthesis and mucosal regeneration (Lobley et al., 2001).

We did not observe an elevation of inflammatory cytokine IL-6 within 48 h following *E. coli* challenge or any treatment effect on IL-6 levels. Similarly, Burkey et al. (2004) did not observe an antibiotic effect or time effect on serum IL-6 within 6 d following an oral gram-negative *Salmonella typhimurium* challenge, although antibiotic was effective in alleviating feed intake depression and febrile response. Interleukin-6 is reported to be associated with hypothalamic-pituitary-adrenal axis modulation in rodents (Rivier, 1995). In the current study, there was no difference in serum ACTH and cortisol among treatments within 48 h *E. coli* challenge. In a previous study of weaned pigs orally challenged with ETEC K88$^+$, the authors also reported no elevation in serum cortisol concentrations at 10 d after the challenge (Jones et al., 2001). Bosi et al. (2004) found that SDP enhanced specific antibody production and decreased inflammatory cytokine expression of orally ETEC K88$^+$-challenged weaned pigs. However, the inflammatory cytokine production and hypothalamic-pituitary-adrenal axis activation of pigs challenged with live *E. coli* was different from other studies using an *E. coli* lipopolysaccharide challenge. Frank et al. (2003) reported that after an i.v. *E. coli* lipopolysaccharide infection, pigs fed a 7% SDP diet had increased serum ACTH, IL-1β, and IL-6 relative to the pigs without feeding a SDP diet. After an i.p. *E. coli* lipopolysaccharide challenge, pigs fed a 7% SDP diet had increased serum

TNF-α and IFN-γ (Touchette et al., 2002), as well as serum ACTH and cortisol (Carroll et al., 2002) compared with pigs fed the diet without SDP. The different immune and hypothalamic-pituitary-adrenal axis responses to pathogen infection might be attributed to the immunogenicity of live *E. coli* vs. *E. coli* lipopolysaccharide, the route of administration, severity of pathogenic infection, differential inflammatory cytokine production, and the specific role of systemic vs. local immune defense system.

At 6 h after the challenge, pigs in all treatments had increased serum GH and decreased serum IGF-1, indicating the uncoupling of GH/IGF-1 axis after pathogenic infection. In agreement with our findings, Wright et al. (2000) reported that after an i.p. *E. coli* lipopolysaccharide challenge, serum IGF-1 of pigs was significantly reduced from 2 to 44 h and reached maximal suppression at 6 h postinfection, which was accompanied by an overall increase in serum GH from 6 to 44 h after challenge. We are not sure why the pigs in the NONC followed a pattern similar to that of GH and IGF-1 response of *E. coli*-challenged pigs. It might be a transitory response of pigs in the NONC to the lateral cross-infection of *E. coli*-challenged pigs, which was also demonstrated in the similar febrile response pattern of pigs across treatments within 48 h following *E. coli* challenge. At 36 h after the challenge, SDP pigs had higher GH and lower IGF-1 concentrations than NONC pigs, which might represent a higher degree of GH/IGF-1 uncoupling. The uncoupling of the GH/IGF-1 axis during *E. coli* K88$^+$ challenge may involve decreased hepatic GH receptor and IGF-1 mRNA expression (Wolf et al., 1996) or disruption of negative feedback of IGF-1 on pituitary GH secretion (Gianotti et al., 1998). At 12 h after inoculation, NONC pigs without *E. coli* infection had higher IGF-1 than *E. coli* K88$^+$-challenged CHAC, SDP, and GLN, indicating the depressing effects of *E. coli* challenge on circulating IGF-1. At 36 postchallenge, NONC pigs had higher IGF-1 than *E. coli*-infected SDP pigs, but not different from that of *E. coli*-infected CHAC and GLN pigs. Within 48 h after the challenge with *E. coli* K88$^+$, only NONC and GLN pigs maintained serum IGF-1 concentrations to the preinfection baseline, whereas CHAC and SDP pigs had decreased IGF-1 compared with the preinfection baseline. Interestingly, the circulating IGF-1 concentrations of pigs across treatments were positively correlated with the growth performance of pigs after 48 h of *E. coli* infection. These results indicate that it is important to maintain the circulating IGF-1 to the preinfection basline levels, and that SDP and GLN may have different modulatory mechanisms on GH/IGF-1 uncoupling after pathogenic *E. coli* challenge. However, this mechanism needs to be further studied in terms of how *E. coli*-challenged pigs in the GLN were able to maintain circulating IGF-1 to the preinfection baseline level, and also to maintain growth performance of NONC pigs without pathogenic infection. It should be noted, however, that limitations of the current experimental design prevent us from determining whether pigs fed SDP and GLN without *E. coli* infection might have similar effects on the measurements collected in this study.

Implications

Feeding glutamine is effective in alleviating growth depression caused by an *Escherichia coli* K88$^+$ challenge. Feeding either spray-dried plasma or glutamine mitigated villus atrophy and intestinal morphology disruption after *Escherichia coli* challenge, indicating the beneficial effects of spray-dried plasma and glutamine in preventing pathogenic infection and maintaining normal intestinal integrity and function. Feeding spray-dried plasma or glutamine may have different modulatory mechanisms on the growth hormone/insulin-like growth factor-1 uncoupling under an *Escherichia coli* challenge situation.

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